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life technologies

Instruction Manual

MultiShot™ StripWell Mach1™-T1R Chemically Competent *E. coli*

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Important Information

Shipping and Storage

The MultiShot™ StripWell Mach1™-T1^R Chemically Competent *E. coli* kit is shipped on dry ice. Upon receipt, store the kit at -80°C.

Contents

The MultiShot™ StripWell Mach1™-T1^R Chemically Competent *E. coli* kit contains the following reagents. Fifty microliters of chemically competent cells are supplied per well with a transformation efficiency of >1 x 10^9 cfu/µg DNA.

Item	Composition	Amount
S.O.C. Medium (store at room temperature or +4°C)	2% Tryptone 0.5% Yeast Extract 10 mM NaCl 2.5 mM KCl 10 mM $MgCl_2$ 10 mM $MgSO_4$ 20 mM glucose	2 x 15 ml
Mach1™-T1^R Cells	---	1 96-stripwell plate
pUC19 Control DNA	10 pg/µl in 5 mM Tris-HCl, 0.5 mM EDTA, pH 8	50 µl

Genotype

F$^-$ ϕ80(*lac*Z)ΔM15 Δ*lac*X74 *hsd*R($r_K^-m_K^+$) Δ*rec*A1398 *end*A1 *ton*A

Product Qualification

- Competent cells are tested for transformation efficiency using the control plasmid included in the kit and following the procedure on page 2. Transformation efficiency should be >1 x 10^9 cfu/µg of pUC19 DNA.
- Untransformed cells are tested for the appropriate antibiotic sensitivity, the absence of phage contamination, and resistance to phage T5 (a standard test that demonstrates resistance to phage T1).

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Important Information, continued

Information for Non-U.S. Customers

For European Customers
The Mach1™-T1^R *E. coli* strain is genetically modified to carry the *lac*ZΔM15 *hsd*R *lac*X74 *rec*A *end*A *ton*A genotype. As a condition of sale, this product must be in accordance with all applicable local legislation and guidelines including EC Directive 90/219/EEC on the contained use of genetically modified organisms.

For All Non-U.S. Customers
The parental strain of Mach1™-T1^R *E. coli* is the non-K-12, wild-type W strain (ATCC #9637, S. A. Waksman). Although the parental strain is generally classified as Biosafety Level 1 (BL-1), we recommend that you consult the safety department of your institution to verify the Biosafety Level.

Methods

Overview

Introduction

The MultiShot™ StripWell Mach1™-T1^R Chemically Competent *E. coli* kit is chemically competent Mach1™-T1^R *E. coli* packaged in a 96-stripwell plate to simplify medium and high throughput bacterial transformation. The stripwell format allows you to use only the number of wells you need for your particular application.

Features of the Strain

The Mach1™-T1^R *E. coli* strain is modified from the wild-type W strain (ATCC #9637, S. A. Waksman) and has a faster doubling time compared to other standard cloning strains. With Mach1™-T1^R cells, you can visualize colonies 8 hours after plating on ampicillin selective plates. You can also prepare plasmid DNA 4 hours after inoculating a single, overnight-grown colony in the selective media of choice. Note that this feature is not limited to ampicillin selection.

The Mach1™-T1^R *E. coli* strain possesses several additional features that make it an ideal strain to use for most cloning applications. These features include:

- *lac*ZΔM15 for blue/white color screening of recombinants
- *hsd*R mutation for efficient transformation of unmethylated DNA from PCR applications
- Δ*rec*A1398 mutation for reduced occurrence of homologous recombination in cloned DNA
- *end*A1 mutation for increased plasmid yield and quality
- *ton*A mutation to confer resistance to T1 and T5 phage

Transforming Competent Cells

Introduction

A procedure is provided below to transform MultiShot™ StripWell Mach1™-T1^R Chemically Competent *E. coli*. Depending on your application of interest, you may modify this procedure to fit your needs.

Important

Do not electroporate chemically competent cells. The salt content of the buffer will cause arcing and kill the cells.

Before Starting

Perform the following before starting the transformation procedure:

- Prepare a container of ice large enough to chill the number of wells you will be using.
- Warm the vial of S.O.C. Medium (supplied with the kit) to room temperature.
- Preheat a water bath to 42°C.
- Warm the selective plates in a 37°C incubator for 30 minutes (use one plate for each transformation). If you are including the pUC19 control, make sure that you have one LB agar plate containing 100 µg/ml ampicillin.

  Note: For optimal growth of Mach1™-T1^R *E. coli* cells, it is essential that selective plates are prewarmed to 37°C prior to spreading.

Transformation Procedure

We recommend including the pUC19 control plasmid DNA supplied with the kit in your transformation experiment to verify the efficiency of the competent cells.

1. Remove a MultiShot™ StripWell plate from the freezer and remove the number of wells you need. Return any unused wells to the freezer. Place the wells in the container of ice. Cells should thaw within 1 minute.
2. Carefully remove the strip of caps from each set of 8 wells and keep them for further use.

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Transforming Competent Cells, continued

Transformation Procedure, continued

3. Use a multi-channel pipet to add 2-5 µl DNA (2 pg-20 ng) to the wells. Keep the volume consistent between wells for uniform results. If you are transforming the pUC19 control, add 1 µl (10 pg) into one well.
4. After adding the DNA, cover the wells with the caps and incubate the cells and DNA on ice for 30 minutes.
5. Transfer the wells to the water bath and heat-shock for 30 seconds at 42°C. **Note**: Be careful not to contaminate the cells.
6. Transfer the wells back to the ice and allow the wells to cool for 1 minute.
7. Remove the caps and add 250 µl of room temperature S.O.C. Medium to each well. **Re-cap the wells tightly.**
8. Incubate the wells at 37°C for 1 hour with shaking (225 rpm). We turn the wells on their side to increase aeration and secure them to the shaker.
9. For each sample, spread 25-100 µl on a **prewarmed** selective plate. Store the remaining transformation mix at +4°C. Additional cells may be plated out the next day, if desired.
10. Invert the plates and incubate at 37°C. If you are using ampicillin selection, visible colonies should appear within 8 hours, and blue/white screening can be performed after 12 hours. If you are selecting transformants with an antibiotic other than ampicillin, incubate plates overnight.
11. Select overnight-grown colonies and analyze by plasmid isolation, PCR, or sequencing. For plasmid isolation, inoculate a single, overnight-grown colony in 2 ml of **prewarmed** selective media (*e.g.* LB + ampicillin, LB + kanamycin, LB + Zeocin™, *etc.*). For optimal results, we recommend inoculating as much of the single colony as possible. Shake at 37°C for 4 hours before isolating the plasmid.

Appendix

Technical Service

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- View and download vector maps and sequences
- Download manuals in Adobe® Acrobat® (PDF) format
- Explore our catalog with full color graphics
- Obtain citations for Invitrogen products
- Request catalog and product literature

Once connected to the Internet, launch your web browser (Internet Explorer 5.0 or newer or Netscape 4.0 or newer), then enter the following location (or URL):

http://www.invitrogen.com

...and the program will connect directly. Click on underlined text or outlined graphics to explore. Don't forget to put a bookmark at our site for easy reference!

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Technical Service, continued

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Notes

Notes

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